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MECHANISMS OF INSECTICIDE RESISTANCE IN COTTON JASSID *AMRASCA BIGUTTULA* (ISHIDA) (HOMOPTERA: CICADELLIDAE)

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ABSTRACT

Cotton jassid, Amrascabiguttula (Ishida) (Homoptera: Cicadelliate) is the key sucking pest of American cotton and has the capability of inflicting heavy losses to the crop Development of resistance in insect pests to insecticides is a natural phenomenon. Realizing the significance of assessing resistance development to insecticides, the experiments to study the mechanism of insecticide resistance revealed that, the activity of general esterases increased through successive 5 generations from 395.833 to 4625.000 n mole of α naphthol formed/min/g of jassid against oxydemetonmethyl and imidacloprid selection pressure. The AchE activity was less than general esterases.

KEYWORDS: Amrascabiguttula, Insecticides, Bioassay, Resistance & Esterases

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Original Article

INTRODUCTION

Cotton is a very important commercial crop for India. It sustains the country's cotton textile industry which is perhaps the largest segment of organised industries in the country. India earns foreign exchange to the tune of $ 10-12 billion from exports of cotton yarn, thread, fabrics, apparel and made-ups. Cotton provides gainful employment to millions of people in the country who are engaged in its cultivation, trading, processing, manufacturing, fabricating and marketing .

India has largest area under cotton (8.7 million hectare) but with quite low yield (10.36 q/ha) a compared to Pakistan (19.86 q/ha), Uzbekistan (22.60 q/ha), USA (25.11 q/ha), Brazil (33.69 q/ha) and Turkey (40.02 q/ha) (Anonymous 2002)

Cotton is attacked by a number of insect pests. Out of 60 insect pest species, significant damage to crop is caused by 10 key pests in most cotton production systems (Luttrell et al 1994). In crop production of cotton from sowing to maturity many insect pests attack in the field, among these sucking pests cotton jassidAmrascabiguttula (Ishida) and whitefly *Bemisia tabci* (Genn) are important (Dhawan and Simwat 2002)

Cotton jassid *Amrasca biguttula* (Ishida) is the key sucking pest of cotton and has been reported to inflict a loss upto 85.45 per cent i.e. 114 kg/ha in seed cotton production (Sidhu and Dhawan 1986). Both nymphs and adults suck cell sap from the leaves and inject toxic saliva into the plant tissue, which initially causes yellowing and curling of leaves at the margins, followed by browning, bronzing, cupping, withering, necrosis and ultimately shedding of the leaves (Hussain and Lal 1940)

Development of resistance to insecticides is generally acknowledged as one of the most serious obstacle to viable insect pest management. The problem has grown tremendously particularly after the introduction of synthetic organic insecticides for the control of insect pests of agricultural, veterinary and public health importance. The first case of resistance was reported in 1914 from USA, when San Jose scale was reported to have developed resistance against lime sulphur by Melander. Since then the number of insect and mite pests reported to have become resistant to one or more insecticides has grown substantially. There are now about 542 species of arthropods reported to be resistant to one or more insecticides in different parts of the would (Whalon 2004). The resistance problems in some areas have become so serious that substantial losses occur in certain vital crops like cotton.

The common practice to manage this pest has been the use of various insecticides especially systemic ones. The sole dependence on insecticides for control of insect pests has led to several problems viz., killing of non target pests including natural enemies, development of insecticide resistance, resurgence, appearance of secondary pests, etc. (Lim 1999)

The insecticide resistance in cotton jassid at several places has been developed due to their excessive and indiscriminate use in the farmers field (Vidyasekhar et al 1989, Sabitha et al 1994, Patel and Yadav 199, Santhini and Uthamasamy 1997) However, any such information available on insecticide resistance on cotton jassid in Punjab is lacking. The present investigation was therefore planned to generate the information about the status of insecticide resistance in cotton jassid keeping in view the following objectives.

MATERIALS AND METHODS

The investigations were carried out in Insect Toxicology Laboratory of the Departmentof Entomology, Punjab Agricultural University, Ludhiana

Biological Materials

Amrascabiguttula (Ihida) (CicadellidaeHomoptera) was collected from Punjab Agricultural University Research Farm and various places of Punjab a given in Table 1. The test insect was subsequently maintained in the screen house at Entomological Research Farm and was used for all experiments as and when required Freshly emerged adults of the test insect were used in the studies.

Insecticides, Chemicals and Reagents

The commercial grade insecticides used in bioassay studies were procured from the local market. The following chemical and reagent were used to study the mechanism of insecticide resistance in cotton jassid through qualitative and quantitative assay of different enzyme responsible for imparting resistance in the pest towards different insecticide employing electrophoresis and UV-VIS spectrophotometer method.

- Tri HCL buffer, 0.2MpH8 (SISC Research Laboratory Pvt.Ltd Bombay)
- Glutathione (GH) (SISC Research Laboratory Pvt.Ltd Bombay)
- I-chloro 2, 4 dinitrobenzene, 0.2 M (CDNB) (SISC Research Laboratory Pvt.Ltd Bombay)
- 0.1M phosphate buffer (SISC Research Laboratory Pvt.Ltd Bombay)
- a-naphthyl acetate (S.D.Fine Chemicals Ltd. Boisar)

- Sodium lauryl sulphate (SISC Research Laboratory Pvt.Ltd Bombay)
- Acrylamide (S.D.Fine Chemicals Ltd. Boisar)
- Bisacrymide (S.D.Fine Chemicals Ltd. Boisar)
- Tri Hcl (pH8.8 and 6.8) (SISCResearch Laboratory Pvt.Ltd Bombay)
- Ammonium per sulphate (SISC Research Laboratory Pvt.Ltd Bombay)
- TEMED (SISC Research Laboratory Pvt.Ltd Bombay)
- Glycine (SISC Research Laboratory Pvt.Ltd Bombay)
- Triton X-100 (SISC Research Laboratory Pvt.Ltd Bombay)
- Sucrose (Qualigens Fine Chemical, Bombay)
- Bromophenol blue (SISC Research Laboratory Pvt.Ltd Bombay)
- Fast blue RR salt SISC Research Laboratory Pvt.Ltd Bombay)
- Methanol (Qualigens Fine Chemical, Bombay)
- Ethanol (Qualigens Fine Chemical, Bombay)
- Agar agar (Qualigens Fine Chemical, Bombay)
- B-naphthyl acetate (SISC Research Laboratory Pvt.Ltd Bombay)
- a-naphthol (SISC Research Laboratory Pvt.Ltd Bombay)

Equipment

In addition to the common laboratory apparatus, the following equipments were also employed.

- Potter tower (Burkard Manufacturing Company Ltd. England)
- Spectrophotometer (Pharmacia Biotech (Biochrom) Ltd.)
- Refrigerated centrifuge
- (Remi Manufacturing Company Ltd.)
- Microsyringe (Labsystems Jag Kumar & Co. Bombay)
- Electrophoresis apparatus (Torsons Code 7020)
- Tissue homogenizer
- Special jassid collection jar
- Special jassid rearing box
- Indigenous temperature control box
- Special Petridish for bioassay

- Incubator
- Foam
- Filter paper
- Aspirators
- Insect collection net
- Split cage
- Micropipettes

Standardization of Bioassay Techniques for Insecticide Resistance Studies on Cotton Jassid

Raising of Cotton Plants

American cotton plants Gossypiumvar LH 1556 were raised in earthen pots (30cmrim diameter) after filling them with mixture of farmyard manure and soil in 1:1 ratio. Sowing of seeds in pots was done in a staggered manner at 10 days interval to ensure continuous supply of required number of plants with similar age and growth for insecticide treatments at different days to carry out various experiments.

Raising of Cotton Jassid Colonies

The colonies of jassid were maintained on cotton, okra, potato plants according to the season. Jassid nymphs and adults were collected from unsprayed cotton fields of Punjab Agricultural University Research Farms with the help of aspirators and net. The collected population was released in split cages as well as in open under screen house conditions to get continuous supply of required number of different stages of cotton jassid for different studies

Rearing of Insect for Generation Studies

The Ludhiana population was collected and exposed to LC75 of test insecticides oxydemeton methyl and imidacloprid by the above standardized bioassay method. The survivors were released in the separate cages for further generation development up to 5 generations. Each generation insect population was collected and subjected to selection pressure. This procedure continued till fifth generation.

Cotton jassid *Amrasca biguttula* was collected from the cages in each generation under controlled conditions. A known number of them and weighing between 30-50 mg were used immediately for kinetic assay of enzymes at optimum pH, and optimum temperature.

General Esterases

Estimation of general esterases was carried out following the method of Wool and Greenberg (1990)

Extraction Procedure

Insects were homogenized in pre-chilled Teflon homogenizer using 3 ml of ice cold 0.1 M phosphate buffer (pH 6.5, having 0.1% triton x-100). The extract was centrifuged at 10,000 rpm for 20 minutes. The supernatant thus obtained was used as enzyme extract for determination of enzyme activity.

Reagents

- 0.1M phosphate buffer (pH 6.)
  - Monosodium dihydrogen phosphate $NaH_2PO_42H_2O$ (0.2M) – 31.20g/l
  - Disodium hydrogen phosphate Na_2HPO_4 $2H_2O$ (0.2M) – 28.30g/l

  Mixed 19.5 ml of A and 30.5 ml of B solution and adjusted volume to 100 ml with distilled water.
- a-Naphthyl acetate (3mM) 5.58 mg of a naphthyl acetate in 10 ml of acetone.
- 0.3% Fast blue B solution.

Dissolved 300 mg of fast blue B dye in 100 ml of 3.5% SDS (3.5% aqueous sodium dodecyl sulphate (SDS) was prepared by dissolving 3.5g of SDS in 100ml of H2O)

Enzyme Assay

The enzymatic reaction was initiated by adding 1 ml of appropriately diluted homogenate in reaction mixture, 0.2 ml substrate solution and 1.7 ml of phosphate buffer and was incubated for 30 min at 37^0C. The reaction was stopped and colour was developed by adding 0.5ml of 0.3% Fast Blue B solution.

The absorbance was read at 602nm using UV-VIS spectrophotometer against reagent blank, containing phosphate buffer substrate solution and Fast Blue B solution after 10 min.

The enzyme activity was calculated from a standard curve prepared by using different concentration of a naphthol. The enzyme activity was expressed in the form of n mole of a-naphthol formed/min/g of cotton jassid.

Acetylcholinesterase

Estimation of Acetylcholinesterase was carried out following the method of Ellman et al (1961)

Extraction Procedure

Insects were homogenized in pre-chilled Teflon homogenizer using 3 ml of ice cold 0.1 M phosphate buffer (pH 6.5, having 0.1% triton x-100). The extract was centrifuged at 10,000 rpm for 20 minutes. The supernatant thus obtained was used enzyme extract for determination of enzyme activity.

Reagents

- 0.1 M phosphate buffer
  - Monosodium dihydrogen phosphate NaH2PO4 2H2O(0.2m) – 31.202g/L
  - Disodium hydrogen phosphate Na2HPO4 (0.2M) – 28.392g/L

  Mixed 39.0 ml of solution A and 61.0 ml of solution B and adjusted the volume 200 ml
- Acteylthiocholine iodide (ATchl) (6nM) Dissolved 16.394 mg of ATchl in 10ml distilled water.
- DTNB (S,S'-dihionitrobis-2(2 nitrobenzoic acid) (0.8 mM) Dissolved 31.7 mg of DTNB in 100 ml of distilled water

Enzyme Assay

0.1ml of homogenate was used for initiating reaction in a reaction mixture containing 1.6 ml of phosphate buffer, 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB and was incubated for 30 min at 37^0C. The absorbance was measured at 405 nm against reagent blank using UV-VIS spectrophotometer after 10 min.

The enzyme activity was calculated from standard curve of reduced glutathione and was expressed in the form of n mole of free thiol group formed /min/g.of insect.

Different generations of cotton jassid collected from entomological research farm were brought to laboratory and their homogenates were subjected to PAGE following the method of Devonshire and Moore (1982)

RESULTS AND DISCUSSIONS

Mechanisms of insecticide resistance in cotton jassid

Kinetic studies of general esterases and Acetylchoiinesterase of cotton jassid

Impact of Oxydemeton methyl on activity of general esterases

Variations occur in general esterase activity towards a naphthyl acetate to different generations of cotton jassid as shown in Table 1.

There was a gradual increase in the activity from first generation to fifth generation. The activity was minimum in 1^{st} generation of 395.833 n mole of α naphthol formed/min/g of cotton jassid and the activity was maximum in 5^{th} generation of 4222.220 n mole of a naphthol formed/min/g of cotton jassid against oxydemeton methyl. The activity of 2^{nd}, 3^{rd} and 4^{th}generations viz. 792.361, and 1981.944 n mole of a naphthol formed/min/g of cotton jassid respectively. The increase of activity was gradual from first generation to fourth generation, however, there was an abrupt increase in the activity from fourth generation to fifth generation.

Impact of Imidacloprid on Activity of General Esterases

Variation was observed in the general esterase activity against Imidaclorprid as shown in Table 2.

The variations are maximum or there was a high activity of general esterases against imidacloprid than oxydemeton methyl. The minimum activitiy of 425.00 n mole of a naphthol formed/min/g of cotton jassid was observed in 1^{st} generation and maximum activity of 4625.000 n mole of a naphthol formed/min/g of cotton jassid was observed in the 5 generation.

The general esterase activity of 2^{nd}, 3^{rd} and 4^{th} generations viz. 1981.944, 2775.000 and 3303.470 n mole of a naphthol formed/min/g of cotton jassid respectively. In first three generations, only 4 isozymes were found i.e. Est 1, Est 2, Est 4 and Est 5, but the remaining 2 generations of fourth and fifth, Est 2 was absent, instead Est 3 was present with Rm value of 0.57.

Impact of Imidacloprid on Acetylcholinesterase

Variations were observed in the acetylcholinesterase activity against Imidacloprid as shown in Table 3.

The activity was low in 1^{st} generation having 194.909 n mole of free thiol formed/min/g of cotton jassid and it was high in fifth generation having 1975.931 n mole of free thiol formed/min/g of cotton jassid. The activity of regaining

2nd, 3rd and 4th generations were 592.710, 1185.420, 1481.776 n mole of free thiol formed/min/g of cotton jassid, respectively. The activity of 5th generation insect is five times more than 1st generation insect but 1.33 times more than 4th generation insect.

Mechanism(s) of Insecticide Resistance

Variations in the activity of general esterases and acetyl cholinesterase were observed in the experiments with different generations of cotton jassid after applying selection pressure. The highest activity of general and acetyl cholinesterases were found in fifth generation insect against oxydemeton methyl and imidacloprid selection pressure. When compared with AchE, the general estareses activity was high in all the generations. The elevated level of general esterases has been correlated with resistance against organophosphate compounds (Guerrero *et al* 1999). The activities of both general and acetylcholine esterases were high when it was selected with imidacloprid than oxydemeton methyl. The activity of AchE was gradually increased from first generation to fifth generation. When the isozymic pattern of general exterases was studied by polyacrylamide gel electrophoresis five isozymes (Est 1 to Est 5) were found. The four isozymes namely Est 1, Est 2, Est 4 and Est 5 were found in first three generations with the Rm value of 0.11, 0.44, 0.62 and 0.68 respectively. But in fourth and fifth generations Est 2 was absent but Est 3 was present with Rm value of 0.57. Usnani and Knowles (2001) found as many as 11 bands of esterase activity in third instars of male and female of *H. zea*$_y$ *S. frugiperda* and *A. ipsilon*species.

There was a high enzyme activity in the fifth generation against oxydemeton methyl having 1086.000 n mole of free thiol formed/min/g of cotton jassid compared to first generation having 177.813 n mole of free thiol formed/min/g of cotton jassid the activity of 2nd,3rd and 4th generations were 296.355, 395.186 and 691.610 n mole of free thiol formed/min/g of cotton jassid respectively.

The activity of fifth generation insect is six times more than first generation insect, but the activity was 1.57 times more than 4 generation insect.

Table 1: Variations in General Esterases Against Oxydemetonmethyl

Generations	Activity (N Mole of α Naphthol Formed/Min/g of Jassid)
I	395.833
II	792.361
III	925.000
IV	1981.944
V	4222.220

Table 2: Variations in General Esterases Against Imidacloprid

Generations	Activity(n mole of α naphthol formed/min/g of jassid)
I	425.000
II	1981.944
III	2775.000
IV	3303.470
V	4625.000

Table 3: Variations in Acetylcholinesterase Against Oxydemetonmethyl

Generations	Activity (N Mole of Free Thiolformed/Min/g of Jassid)
I	177.813

Table 3: Contd.,	
II	296.355
III	395.186
IV	691.610
V	1086.000

Table 4: Variations in Acetylcholinesterase Against Imidacloprid

Generations	Activity (N Mole of Free Thiol Formed/Min/g of Jassid)
I	194.909
II	592.710
III	1185.420
IV	1481.776
V	1975.931

Table 5: Impact of Oxydemetonmethyl on activity of general esterases, AchE and susceptibility

Generation	General Esterase Activity (N Mole of α Naphthol Formed/Min/G of Jassid)	Acetylcholinesterase Activity (N Mole of Free Thiol Formed/Min/g of Jassid	LC_{50}(%)
1	395.833	177.813	0.00518
2	792.361	296.355	0.00665
3	925.000	395.186	0.00783
4	1981.944	691.610	0.00857
5	4222.222	1086.000	0.00995

Table 6: Impact of Imidacloprid on Activity of General Esterases, Ache and Susceptibility

Generation	General Esterase Activity (N Mole of α Naphthol Formed/Min/G of Jassid)	Acetylcholinesterase Activity (N Mole of Free Thiol Formed/Min/G of Jassid	LC_{50}(%)
1	425.000	194.909	0.000500
2	1981.944	592.710	0.000620
3	2775.000	1185.420	0.000765
4	3303.470	1481.776	0.000895
5	4625.000	1975.931	0.000963

CONCLUSIONS

In the experiments to study the mechanism of insecticide resistance, population was collected from Ludhiana and was reared in the cages for five generations with selection pressure of oxydemeton methyl and imidacloprid. Each generation was selected at LC75 and the survivors were allowed to multiply on untreated plants. The impact of insecticides on the activity of general esterases and acetylcholineterase were calculated. The general esterases activity increased through successive generations. The activity against oxydemeton methyl from first generation to fifth generation was 395.833, 792.361, 925.000, 1981.944 and 4222.22 n mole of α-naphthol formed/min/g of jassid. Respectively but against imidacloprid, it was 425.000, 1981.944, 2775.000, 3303.470 and 4625.000 n mole of α naphthol formed/min/g of jasid. The acetylcholinesterase activity was less than general eterases ranging from 177.813 to 1086.000 n mole of free thiol formed/min/g of jassid against oxydemeton methyl and 194.909 to 197.931 n mole of free thiol formed/min/g of jassid against imidachloprid.

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